Serum levels of endostatin, vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) in patients with acute myocardial infarction undergoing early reperfusion therapy

Yoshinori SEKO*, Shuichi FUKUDA† and Ryozo NAGAI*
*Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan, and †Division of Endocrinology and Metabolism, Jichi Medical School, Tochigi, Japan

ABSTRACT

Angiogenesis is controlled by anti-angiogenic factors as well as by angiogenic factors, such as VEGF (vascular endothelial growth factor) and HGF (hepatocyte growth factor). Endostatin, a potent endogenous angiogenesis inhibitor, is known to inhibit endothelial proliferation and suppress tumour growth. However, to date, little is known about the pathophysiology of endostatin in ischaemia/reperfusion. To investigate the mechanisms of angiogenesis induced by myocardial ischaemia/reperfusion in more detail, we studied the circulating levels of endostatin, VEGF and HGF in 17 patients with acute myocardial infarction, who underwent early reperfusion therapy. In all patients, serum endostatin, VEGF and HGF levels before reperfusion were increased significantly compared with those in 17 control subjects (endostatin, 49.2 ± 11.7 ng/ml, but not detectable in controls; VEGF, 685.6 ± 150.3 pg/ml compared with 173.7 ± 33.6 pg/ml; HGF, 3638 ± 1285 pg/ml compared with 59 ± 13 pg/ml; values are means ± S.E.M.). After reperfusion, the serum endostatin and VEGF levels decreased significantly, but still remained higher than those in control subjects (endostatin, 19.6 ± 7.0 ng/ml; VEGF, 284.2 ± 90.2 pg/ml). In contrast, serum HGF levels increased significantly (15 146 ± 2230 pg/ml) after reperfusion. These data indicated that serum levels of endostatin changed in parallel with those of VEGF in response to myocardial ischaemia/reperfusion, and the marked increase in serum HGF levels after reperfusion seemed to be, at least in part, due to heparin administration. Our data offer a possible anti-endostatin therapy in patients with acute myocardial infarction to facilitate collateral vessel formation.

INTRODUCTION

Angiogenesis is thought to be controlled by the net balance between anti-angiogenic factors and angiogenic factors, such as VEGF (vascular endothelial growth factor) and HGF (hepatocyte growth factor). We reported previously [1] that serum levels of VEGF in patients with acute myocardial infarction were markedly increased and rapidly returned to normal levels by early reperfusion, indicating that circulating levels of VEGF reflect myocardial ischaemia. However, little is known about the pathophysiology of anti-angiogenic factors in ischaemia/reperfusion.

Endostatin, a 20 kDa C-terminal fragment of collagen XVIII, was isolated from murine haemangiendothlioma and has been shown [2] to potently inhibit endothelial cell proliferation. In vivo administration of endostatin resulted in a strong suppression of tumour-induced
Table 1  Characteristics of the patient and control groups

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>17/0</td>
<td>17/0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.2 ± 2.7</td>
<td>56.9 ± 2.1</td>
</tr>
<tr>
<td>Smoking (n)</td>
<td>15 (88.2)</td>
<td>6 (35.3)</td>
</tr>
<tr>
<td>Hypertension (n)</td>
<td>7 (41.2)</td>
<td>7 (41.2)</td>
</tr>
<tr>
<td>Diabetes mellitus(n)</td>
<td>8 (47.1)</td>
<td>8 (47.1)</td>
</tr>
<tr>
<td>Hyperlipidaemia (n)</td>
<td>4 (23.5)</td>
<td>4 (23.5)</td>
</tr>
</tbody>
</table>

angiogenesis [2]. Although many studies have reported the circulating levels of endostatin in patients with various tumours, there have been no reports studying the role of endostatin in acute ischaemia or reperfusion. In the present study, we report the circulating levels of endostatin, VEGF and HGF in patients with acute myocardial infarction undergoing early coronary reperfusion therapy.

METHODS

This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association, and was approved by the Ethics Committee of the University of Tokyo Hospital. All patients gave written informed consent after full explanation of the purpose, nature and risk of all procedures used.

Seventeen patients (17 males; age, 57.2 ± 2.7 years; mean ± S.E.M.) with acute myocardial infarction were studied. The characteristics of the patient and control groups are summarized in Table 1. All of the patients underwent successful early reperfusion therapy. The serum samples before reperfusion were taken without heparin administration, whereas those after reperfusion were collected after the administration of heparin, nitroglycerin, nicorandil and ANP (atrial natriuretic peptide).

Serum endostatin concentrations were measured by using a competitive enzyme immunoassay (Accucyte Human Endostatin™; Cytimmune Sciences, College Park, MD, U.S.A.) with a polyclonal antibody specific for endostatin and a biotinylated endostatin protein (competitive ligand). Serum VEGF and HGF concentrations were measured by a quantitative sandwich ELISA (BioSource International, Camarillo, CA, U.S.A.) with polyclonal or monoclonal primary and secondary antibodies specific for VEGF or HGF. According to the manufacturers, the sensitivity of the assays were 1.95 ng/ml for endostatin, < 5 pg/ml for VEGF and 10 pg/ml for HGF. Serum levels of creatine kinase activity were also measured by the Rosalki method (normal range, 15–168 IU/l; where IU is international units) in these patients.

![Figure 1](image1.jpg)  
Figure 1  Serum levels of endostatin and VEGF in patients with acute myocardial infarction before and after the reperfusion therapy

Individual values and means ± S.E.M. (n = 17) are shown. *P = 0.0003 and †P = 0.0001 compared with before reperfusion, as determined by a paired Student t test.

Values are presented as means ± S.E.M. A paired Student t test was used to evaluate differences in serum levels of endostatin, VEGF, HGF and creatine kinase before and after reperfusion. An unpaired Student t test was used to evaluate differences in serum levels of endostatin, VEGF and HGF between the acute myocardial infarction and control groups.

RESULTS

The serum endostatin level in control subjects was not detectable, but VEGF and HGF concentrations in the controls were 173.7 ± 33.6 pg/ml and 59 ± 13 pg/ml respectively.

Figure 1 shows the concentrations of serum endostatin and VEGF in patients with acute myocardial infarction before and after reperfusion therapy. In all patients except one, whose creatine kinase activity was not markedly elevated after reperfusion, serum endostatin and VEGF levels before reperfusion (49.2 ± 11.7 ng/ml and 685.6 ± 150.3 pg/ml respectively) were increased markedly compared with those in healthy control subjects. After reperfusion for 3–6 h (4.06 ± 0.26 h), the serum endostatin and VEGF levels in these patients decreased significantly (endostatin, 19.6 ± 7.0 ng/ml; P = 0.0003; VEGF, 284.2 ± 90.2 pg/ml; P = 0.0001), but still remained higher than those in control subjects.

The serum HGF concentrations and creatine kinase activities in these patients with acute myocardial infarction before and after reperfusion therapy is shown in Figure 2. In nine patients, serum HGF levels were markedly elevated, although only slightly elevated in the other eight patients before reperfusion, compared with those in control subjects (3638 ± 1285 pg/ml in patients and 59 ± 13 pg/ml in controls). However, the
Serum levels of HGF and creatine kinase in acute myocardial infarction before and after reperfusion therapy.

Individual values and means ± S.E.M. (n = 17) are shown. ‡P < 0.0001 compared with before reperfusion, as determined by a paired Student t test.

On the other hand, serum HGF levels in patients with acute myocardial infarction were significantly elevated compared with control subjects, although some patients did not have elevated levels. Serum HGF levels were significantly increased by early reperfusion. Significant elevation of serum HGF levels in patients with acute myocardial infarction has also been reported in other studies [6–9]. In the present study, the pattern of changes in serum levels of HGF and those of creatine kinase before and after reperfusion was similar (Figure 2). The good correlation between serum levels of HGF and those of creatine kinase in the early stage of acute myocardial infarction is supported by a previous study [6]; however, there was no significant correlation between serum levels of HGF and creatine kinase in the present study.

In the present study, we found a further and marked elevation of serum HGF levels after reperfusion, although this was thought to be, at least in part, due to heparin administration. Further studies using animal models without heparin administration are needed to investigate the precise effect of reperfusion on serum HGF levels. Kitta et al. [10] reported that HGF, at concentrations that can be detected in the sera of patients with acute myocardial infarction, protected cardiac myocytes against oxidative stress-induced apoptosis. Therefore we could not exclude a possibility that HGF may play a protective role in the reperfusion-induced oxidative stress, as well as in ischaemia-induced collateral vessel formation. Among the drugs, other than heparin, which were administered after reperfusion, ANP has been reported to inhibit both the synthesis and function of VEGF [11,12]. Therefore ANP administration after reperfusion might play, at least in part, a role in decreasing serum VEGF levels.

DISCUSSION

In the present study, we have shown clearly for the first time that serum levels of the anti-angiogenic factor endostatin were markedly increased in patients with acute myocardial infarction, but were decreased significantly by early reperfusion. The pattern of changes in serum levels of endostatin and VEGF in patients with acute myocardial infarction before and after reperfusion were similar (Figure 1). This suggested that circulating levels of endostatin and VEGF might be similarly regulated in response to ischaemia and reperfusion. Endostatin has been shown to inhibit VEGF-induced endothelial cell migration and, thereby, suppress tumour growth as well as to induce endothelial cell apoptosis [3,4]. Thus VEGF and endostatin are released into the peripheral circulation in response to ischaemia and may function as facilitating and counteracting factors respectively, for angiogenesis. Feldman et al. [5] reported that circulating levels of endostatin in patients with renal cell carcinoma were elevated and correlated with those of VEGF and were associated with tumour aggressiveness. This suggests that a similar mechanism is involved in the regulation of endostatin and VEGF in patients with tumours and ischaemia.

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REFERENCES


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